CAPILLARY ELECTROPHORESIS FLOW CONTROL SYSTEM

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates generally to capillary electrophoresis and, more particularly, to systems for controlling the bulk flow in capillary electrophoresis.

2. State of the Art

Electrophoresis is well known as an analytical technique for separating and detecting constituents in a sample. Electrophoretic techniques are based upon the fact that each molecular species has a unique combination of mass, size, shape, charge, density and sub-unit structure, all of which may result in mobility differences responsive to an electric field. Various electrophoretic techniques use one or more of these properties to cause varying degrees of separation via the migration of molecular species in the presence of an electric field. Applications for electrophoresis include the determination of sample purity, the determination of molecular weights for proteins and nucleic acids, the mapping of nucleic acid primary structure (e.g., DNA and RNA sequence analyses) and the definition of phenotypic variance of a protein at the molecular level.

Capillary electrophoresis is an electrophoretic technique that employs a capillary tube which is filled with a conductive fluid. In practicing capillary electrophoresis, a small quantity of sample is introduced at one end of the capillary tube, and a potential difference is applied across the ends of the tube. Then, under the influence of the potential difference, electroosmotic flow and differences in electrophoretic mobilities combine to provide a spatial separation of constituents of the sample solution. That is, when a positive electrode is applied to the inlet end of the capillary tube and a ground electrode is applied to the outlet end, spatial separation can be achieved, for example, with positively charged constituents exiting first, followed by neutral constituents and then negatively charged constituents. Each constituent of a sample can be detected by identifying the time required for the constituent to travel through the capillary tube.

Electroosmotic flow is the movement of a liquid relative to a stationary charged surface as a result of an electric field applied to the liquid. It has been explained that electroosmotic flow is a result of charge accumulation at the capillary surface due to chemical equilibrium of the interior surface of the capillary and the electrolyte. The charge of the surface attracts a thin layer of oppositely charged electrolyte ions, which accumulate adjacent to the inner surface. The longitudinally extending electric field that is applied across the capillary tube accelerate the positive ions which are hydrated by water toward a grounded outlet end of the capillary tube, viscously dragging other hydrated molecules. The result is a bulk flow of the sample in the buffer solution toward the grounded outlet end of the capillary tube. Consequently, electroosmotic flow provides a means for moving neutral and negatively charged constituents of a sample toward a ground electrode.

Electrophoretic migration is the movement of charged constituents in response to an electric field. Thus, under the influence of an electric field, a positively charged molecule will be accelerated through the fluid toward the cathode. Under the same circumstances, negatively charged molecules are repelled by the cathode, but the force of the electroosmotic flow may overcome the repulsion and advance the negatively charged molecules.

In practice, the quantity of a constituent within a sample can be determined by the area of a signal trace of an electropherogram during a period of detection of that constituent. Such detection is usually accomplished by placing ultraviolet detectors at the outlet end of the capillary tube, but other placement and detectors are known. In such systems, plate height is a measure of the sharpness of the flow front as measured by the shape of the sample signal. A lower plate height corresponds to a sharper flow front. In general, it is desirable to have a small plate height since plate height is inversely related to the resolution of a capillary electrophoresis system.

In summary, it is known that the voltage difference in a capillary electrophoresis system, when applied to charged molecules, moves these molecules through the system. This phenomenon is known as electrophoretic flow or electrophoretic migration. Electroosmotic flow, on the other hand, is a bulk flow phenomenon in that this is when the solution moves from one end of the capillary electrophoresis system to the other. Electroosmotic flow is a function of the capillary surface charge and the voltage difference, among other factors. In practice, varying electroosmotic flow is one means of controlling bulk flow but it is dependent on the chemistry of the system in use. Furthermore, electroosmotic flow alone cannot be controlled over a range of velocities as easily as pressure. For example, for a capillary with a fused silica surface at pH 7, the electroosmotic flow cannot be adjusted by external means independent of the electrophoretic migration.

What is needed is a method for controlling bulk flow which can be applied over a range of velocities. Furthermore, such a method should be independent of chemistry to permit its application to many situations.

SUMMARY OF THE INVENTION

Generally speaking, the present invention provides a capillary electrophoresis system for controlling bulk flow over a range of velocities and independent of chemistry while maintaining a sharp flow front. More particularly, the present invention provides a system for controlling the bulk flow rate in capillary electrophoresis by employing pressure to adjust velocity without unacceptably increasing plate height. Thus, the system controls bulk flow over a range of velocities, independent of the chemistry of the system.

The present invention employs a pressure differential between the inlet and outlet ports of the capillary which pressure differential can vary or remain constant over the duration of the electrophoretic run, to drive bulk flow in one direction or another. The pressure can go in the same or opposite direction to either the electrophoretic flow or the electroosmotic flow, depending on whether the goal is to increase or decrease the time period during which the charged particles stay in the capillary. The inlet port pressure could be generated by an air pump, while the outlet port is at atmospheric pressure. The pressure differential can encourage flow with or against the electroosmotic flow.

As previously discussed, plate height is a measure of the sharpness of the flow front as detected by a detector, where a lower plate height corresponds to a sharper flow front. Although differential pressure has been avoided in capillary electrophoresis because it degrades resolution and separation, it was found that the use of pressure in conjunction with electroosmotic flow causes only a minimal increase in plate height over that of electroosmotic flow alone, while giving the user considerable control over the bulk velocity. Thus,